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TWO SIMPLE, TIME-SAVING TECHNIQUES for studies of SOIL MICRO-ORGANISMS[1]

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With the variety of highly sophisticated instruments now available for research, inexpensive materials are easily overlooked and are often not even reported in the literature. We have devised some simple techniques that greatly reduce the time and tedium of standard procedures in research on soil micro-organisms.

SPREADING SOIL DILUTIONS ON AGAR PLATES

After samples of soil or the rhizosphere are taken and diluted by traditional methods, aliquots of the diluted suspension can be spread over the surface of hardened agar plates for counting or isolating pure cultures of soil micro-organisms.[2] Sterile glass rods, bent at right angles into a loop with a flat side, serve well to spread the suspension evenly (fig. 1). The ease and efficiency of this operation can be markedly increased, moreover, if the agar plates are revolved on a turntable while the rod is held in a fixed position, rather than the rod itself being moved.

[1] Text is derived from "Two simple, time-saving techniques for studies of soil microbial populations and subsequent culture characterization" by the same authors, published in Soil Biology--Int. News Bull., Comm. III, Int. Soc. Soil Sci., No. 6, p. 38. Dec. 1966. The information is being reissued in this note so that it will be available to a wider audience and illustrations can be included.

[2] Johnson, Leander F., Curl, Elroy A., Bond, John H., and Fribourg, Henry A. Methods for studying soil microflora-plant disease relationships. 178 pp. Minneapolis: Burgess Publ. Co. 1959.

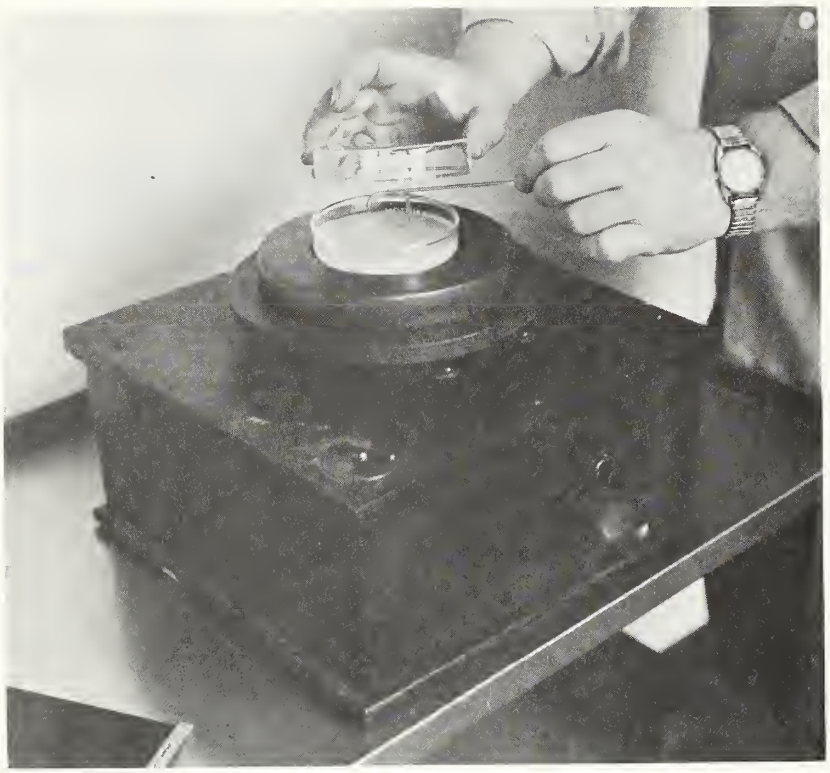

Figure 1.--Spreading an aliquot of soil-water suspension on agar surface with aid of glass rod and modified spring-operated phonograph turntable.

A variable speed phonograph has proved ideal for this purpose (fig. 1). A circular, velvet-lined, wooden mount was fashioned with a lip 7 mm. high to hold a standard-sized petri dish. The mount was attached to the spindle of the phonograph turntable.

In use, an inoculated plate is placed on the turntable, the phonograph motor switched on, and the rod held still against the agar surface. As the petri dish revolves under the rod, the suspension is spread evenly over the agar surface with minimal effort. When hundreds of plates are being spread, this technique spares both time and sore wrists! A hand-operated petri dish spinner is now commercially available.

Before use, the agar plates should be dried 8-12 hours at 37° C. to minimize spread of motile bacteria over the agar surface after inoculation. For best results, the liquid inoculum should be placed near the center of the plate.

ISOLATING PURE CULTURES FROM DILUTION PLATES

Once colonies develop on the agar surface of dilution plates, further time can be saved by using pointed, white birch[3] toothpicks for obtaining pure cultures of individual organisms. The toothpicks are autoclaved in a petri dish and removed from the sterile dish with sterile tweezers as needed to probe a colony on the agar surface (fig. 2). The whole toothpick is then immersed in a tube of enriched nutrient broth, which is then stirred with a vortex mixer and incubated. After suitable incubation, aliquots are examined for purity and transferred to the rapid replicator[4] for study of the organism's physiological characteristics.

When hundreds of organisms are under simultaneous study, as is often the case in extensive research on the rhizosphere, these simple techniques and low-cost materials can yield large dividends in saved time and reduced fatigue.

Figure 2.--Probing a surface colony with a sterile toothpick for isolation of a micro-organism from a dilution plate.

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[3] White birch is not necessary, but woods such as cedar may be somewhat toxic to bacteria.

[4] Neal, J. L., Jr., Lu, K. C., Bollen, W. B., and Trappe, J. M. Apparatus for rapid replica plating in rhizosphere studies. Appl. Microbiol. 14: 695-696, illus. 1966.

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